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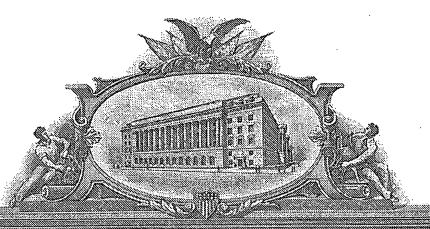
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## HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

### PROVISIONAL APPLICATION FOR PATENT COVER SHEET

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[ ] Additional inventors are being named on the separately numbered sheet(s) attached hereto							
	1	TITLE OF THE INV	ENTION (500 chai	racters max)			
METHOD AND SYSTEM FOR AQUACULTURE PRODUCTION							
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#### METHOD AND SYSTEM FOR AQUACULTURE PRODUCTION

#### **BACKGROUND OF INVENTION**

In 2001, \$12.7 billion of seafood was consumed in the United States. Domestic consumption of these aquatic products increased, on average, 5.1 percent annually from \$8.3 billion in 1992. This recent growth in the consumption of seafood in the United States is significant compared with the 3.2 percent growth in consumption of all nondurable goods. In August, 2003, the Economist magazine predicted 30 more years of growth in the global aquaculture industry. If the following trends continue, the U.S. aquaculture industry will see increasing growth during this time relative to other countries as farms continue to intensify their capital structure:

- the U.S. population continues to grow at approximately 1 percent per year,
- consumers increasingly turn to seafood for its published health benefits,
- consumers preferences change towards seafood as per capita wealth increases over time,
   luxury markets receive new exotic fish, traditional markets become familiar with
   existing seafood products
- fish prices continue to fall through technological advances in aquaculture as well as increased supply, and,
- the global supply of aquatic products from capture fisheries continues to decrease due to over-fishing and environmental degradation.

U.S. aquaculture firms produced \$800 million worth of aquatic products in 2001, enough to meet 6.1 percent of domestic demand.<sub>4</sub> U.S. capture fisheries produced \$5 billion, the equivalent of 39 percent of U.S. demand.<sub>5</sub> Finally, in 2001, \$10.4 billion in seafood was imported into the United States, enough to meet 81.3% of our demand.<sub>6</sub> \*

Aquaculture production in the United Stated has averaged 10.3 percent annual growth since 1984, while capture production has already declined to 1.1 percent. As stocks of known fishery products decline, fisherman may continue to increase annual output by rotating their capture to new replacement species, but this technique is rapidly approaching finitude as the number of potential fishery products approaches zero.

Globally, \$155.1 billion in aquatic products were produced in 2001.<sub>8</sub> \$61.5 billion, or 40 percent, came from aquaculture.<sub>9</sub> While \$93.7 billion still came from world capture fisheries, most industry professionals anticipate this proportion will shift over time.<sub>10</sub> By 2020, 70 percent of global seafood production will most likely come from aquaculture.<sub>11</sub> By maintaining current production and consumption trends, aquaculture will become a trillion dollar global industry by 2030.<sub>12</sub> The retail value of aquaculture's products might then exceed \$3.5 trillion, maintaining the current 200 percent markup over wholesale prices on commodities like salmon.

However, it is important to consider two points:

- International competitors possess tremendous cost savings in terms of labor, land, and inputs.
- Both higher value products such as sashimi-grade bluefin tuna, and niche products like caviar benefit from the controls capital-intensive systems provide.

Without a comparative advantage in resource costs, American aquaculture companies must develop technologies to diminish operating costs and produce superior products. Therefore, U.S. firms are often more competitive when they produce products requiring more capital-intensive systems. Still in its infancy, the U.S. aquaculture industry lags world leaders like Norway and Japan in terms of technological development. Norwegians pioneered intensive salmon farming, which now resembles a mature industry. Chile continues to steal market share of world salmon

production because of cost savings and optimal water conditions. For Chilean farms, the salmon industry is still growing rapidly. For American farmers to develop businesses in anticipation of future industry trends, they must establish themselves in aquaculture sub-sectors in which consumers demand the freshest, high-value products, at competitive prices. Close proximity to market enables optimal freshness, vital for premium pricing, and reduces shipping costs.

U.S. caviar and oyster farms demonstrate that demand exists for locally produced superior goods and that the firms producing these goods can out-compete international producers based on both quality and cost. Caviar farms leverage a technological advantage to produce a good on par with Beluga, and oyster farms exploit location and resource price to offer higher quality, fresher goods, at competitive prices. Because raw seafood intensifies the need for freshness, specific taste, and consistency, intensive farms near their markets enjoy sufficient comparative advantages to succeed even when competing with lower-cost producers in another country. Further, in products like caviar, higher prices are not always a deterrent. Capital-intensive systems in close proximity to markets currently provide superior quality, sometimes lower total costs due to shipping, but usually not lower production costs.

#### SUMMARY OF INVENTION

By transforming system by-products into inputs to be used farther along in the production process, potentially storing these inputs, and, if necessary further refining these inputs prior to a monitored and controlled reintroduction, the present approach is directed to:

- 1. Minimize costs, adjust output to a profit-maximizing level, and optimize any or all of the following: quality, taste, and appearance.
- 2. Allow a firm to base operations producing aquatic products in close proximity to markets, relative to non-recirculating systems.
- 3. Manage quality control and refine appearance/taste of final products based on changing consumer preferences.
- Control production parameters to minimize production costs and stabilize water chemistry as well as other biological factors.

Capitalize on economies of scale and scope by culturing a greater volume of each
production species and greater number of species than would otherwise be economically
feasible without this system.

The present aquaculture production method and system addresses the challenges fish farmers face—how to create an environment that profitably produces an aquatic species of desired quality and quantity. This result is achieved by converting system by-products into inputs. Specifically, nitrogenous wastes are converted into fertilizer for phytoplankton, and carbon dioxide is removed from main production systems and added to phytoplankton production systems.

The following defines an intensive re-circulating aquaculture system. Advantages of the invention include the ability to maximize operating profits and increase control over product quality, taste, appearance. Aquatic product specifications differ based on the type and intended use. For example, finfish produced for human consumption must meet taste, appearance, and nutritional composition standards, should be free from parasites and not contain undesirable chemicals. Industrial products may need to meet a particular chemical composition or structural standard. Ornamental products may primarily need to meet appearance standards, but implied in that appearance is health of the organism to ensure survival.

These goals are achieved by producing feed onsite, by separating major systems like feed production, grow-out, and filtration, and by removing and refining system variables like carbon dioxide and live feed before re-introducing them as inputs in other locations within the system. The system recycles nutrients and converts toxic by-products into production inputs in an attempt to simulate natural ecosystems. While the physical plant will not precisely duplicate natural conditions, separating systems exchanges some aspects of the natural environment for increased product control.

The degree of system intensity, from extensive, to semi-intensive, and finally to intensive, is based on the amount of water used to produce a given mass of aquatic specie and the amount of supplemental feed added to enhance growth rates. Re-circulating refers to systems almost exclusively indoors in which water is re-used after first filtering in order to remove physical

waste and convert toxic dissolved waste by-products into benign forms. Ideally, re-circulating intensive systems eliminate problems outdoor (non-re-circulating) farmers face under any form of system intensity. These problems include reduced control over final product and water quality, unpredictability and presence of adverse environmental factors, predation, and increased contamination from parasites.

A goal of the invention is to maximize the quality of the final product while minimizing feed production costs. Unlike many re-circulating systems, the primary goal of this invention is not to minimize the amount of water used. However, water is conserved more than such non-re-circulating intensive systems as salmon cage culture, and shrimp and catfish pond culture. Other methods attempt to reduce feed inputs while conserving water, e.g., US2003/0154926. However, no known prior art combines methods to maximize profits through all of the following:

- Reduction of feed costs achieved by producing feed internally,
- reduction of waste removal and fertilizer input costs by converting waste and byproducts into inputs
- maximized output and quality achieved by optimizing nutrition and the production environment, and
- Increased controls over product type, taste, quality, and volume achieved by system separation between such systems as product production (grow-out), filtration, and feed production.

As is the case with known aquaculture systems, the idea of integrating product production, filtration, and feed production within one farm dates back thousands of years to China. However, the present approach of integrating these concepts into an industrial system that separates subsystems into distinct divisions, removes and/or refines, then re-introduces organism and chemical solid, liquid, and gas inputs and by-products is novel.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

Figure A illustrates an embodiment of an aquaculture production system in accordance with principles of the present invention.

Figure B illustrates the main production sub-system from figure A excluding the algae (phytoplankton) and zooplankton sub-systems in accordance with the present invention.

Figure C illustrates the algae sub-system from figure A excluding the main production and zooplankton sub-systems.

Figure D illustrates the zooplankton sub-system from figure A excluding the algae and production sub-systems.

#### DESCRIPTION OF INVENTION

The present approach is adaptable to the production of either a single species or multiple species, in fresh, brackish, or saltwater. In order to clearly illustrate the key aspects of the invention, a first embodiment is described for a single species, freshwater finfish farm. However, system parameters are optimized through economies of scale in multi-species, multi-phyla farms. Therefore, other embodiments of multi-phyla farms depict more common uses of this invention. Thus, while the invention is particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein (e.g., surrounding physical plant designs, water quality parameters, and feed species may differ) without departing from the scope of the invention.

#### Key Aspects Common to All Systems:

- 1. Production sub-system (Figure B) for growing final product species from pre-market to market size (primarily finfish, may also be mollusks, crustacean, algae, other)
- Phytoplankton sub-system (Figure C) producing one or more species of algae or diatoms
  (for D. Pulex: Chlorella, Chlamydomonas, Scenedesmus, heterotrophic
  microflaggelates and bacteria). Algae and diatoms are selected based on the
  nutritional requirements and feeding preferences of final product species and zooplankton
  species.
- 3. Zooplankton sub-system (Figure D) for producing one or more species of live feed capable of consuming phytoplankton (moina, daphnia, artemia, copepods, feeder fish, zebra mussels). Phytoplankton are selected based on nutritional requirements and feeding preferences of final product species, as well as ease of culture, reproductive rate, and growth rate.
- 4. Feed processing sub-system that either distributes live feed to production sub-system (1) or refines live feed into pellets and may add nutritional supplements to pellets before feeding to production sub-system (1)
- 5. Filtration units/sub-systems for production sub-system(s) (1), phytoplankton sub-system(s) (2), and zooplankton sub-system(s) (3)
- Solid waste removal and water treatment sub-system that refine solid and liquid wastes
  before either these wastes or used water leave the facility in keeping with environmental
  regulation, pollution licenses and permits
- 7. Product processing sub-system that harvests and processes the final product species in preparation for distribution to markets
- 8. Information technology sub-system that monitors and responds to water quality parameters and production levels of sub-systems 1, 2, 3, 5, 6
- Water storage sub-system for treating and storing water from both external sources and other culture sub-systems before adding to any culture systems
- 10. Ozone sub-systems, UV sub-systems, or other methods for purifying water at a chemical, biological, and physical level may be used
- 11. Micro-culture and live feed culture storage systems for seeding phytoplankton subsystem (2), zooplankton sub-system (3), or biological filtration sub-systems (rotifers)

12. Optional broodstock sub-system for breeding, larval rearing for stocking in production or zooplankton

Single-Species Freshwater Finfish Embodiment

This system may be used to produce most freshwater finfish species. The following example describes a system using comet goldfish as the production species, Chlorella and Chlamydomonas as phytoplankton, and daphnia pulex as the zooplankton. Moina and daphnia magna may also be used. These species are chosen because their small size and availability makes small scale demonstration of the invention easy to duplicate.

#### 1. Production sub-system (Figure B)

The final production species, in this case goldfish, are cultured in the production sub-system (1). They may be grown from juvenile to adult, larva to juvenile for sale to other businesses, or any other size and age range to meet the demands of some pre-determined consumer. This system includes a culture tank, mechanical filter, biological filter, devices for controlling inflow and outflow, and probes in the culture tank and biological filter to measure water quality parameters. As fish grow they may be divided in number and moved into more than one culture tank to increase growth and survival rates by increasing water volume per fish. Fish enter this system as juveniles, which may be purchased from an outside distributor, or raised onsite through a breeding program in a hatchery sub-system (12). The fish are harvested when determined to have met market size.

The culture tank may be of a circular, conical, raceway, doughnut or other design. For this example, a circular tank is used. In all cases water flows through the culture tank and is filtered mechanically and biologically. Solid waste may be removed through mechanical filtration and processed in the solid waste-removal subsystem (6). Much water is initially returned to the production sub-system (1) after biological filtration except for water used to flush solid

waste to the waste removal sub-system (6). The purpose of biological filtration is to convert toxic waste-products such as urea and ammonia to nitrite, and then to nitrate. The process of ammonification and nitrification reduces toxic nitrogenous compounds to non-toxic forms. However, changes in carbon dioxide and pH levels may revert non-toxic forms back to toxic forms. Therefore, water may be automatically removed from the final production sub-system (1) to the phytoplankton sub-system (2) and/or waste removal sub-system (6) should this occur.

Water quality parameters may be monitored within the final production system (1) by the information technology sub-system (8) and other methods. The following parameters among others may be monitored: water flow rate, temperature, dissolved oxygen, carbon dioxide, ammonia, nitrite, nitrate, pH, turbidity, ozone, hardness, salinity. For goldfish, these parameters may be optimized at the following levels:

- pH=7.4
- Total Alkalinity=100 ppm stabilize pH, General Hardness=115
- ammonia<0.1ppm, nitrite<0.2ppm, nitrate<50ppm
- CO2<1ppm
- DO>8ppm
- Temp=20-22C
- copper<0.1ppm
- Salinity: 0.1%-0.3%
- Chlorine<0.5ppm

At any time water may be added from the storage sub-system (9) to correct for adverse conditions. Further, the information technology subsystem (8) may be linked to devices capable of correcting water quality parameters, such as low dissolved oxygen. In this case, the information technology subsystem (8) may detect low dissolved oxygen levels in the main production system (1) by means of a dissolved oxygen probe, and release oxygen through submerged gas distribution tubes until oxygen levels return to optimal levels.

When the information technology sub-system (8) detects that nitrogenous compounds (dissolved salts and gases) have reached a pre-determined threshold, the water in the production sub-system (1) is released into the phytoplankton sub-system (2). Water levels in the production system (1) may be maintained with water inflows from the water storage system (9).

In this example, water may be filtered *mechanically* to remove physical particles, biologically to convert nitrogenous by-products to non-toxic forms as well as organic matter into useful carbon-based by-products, ozone treatment to destroy harmful micro-organisms and parasites as well as further break down organic and inorganic compounds, and finally carbon dioxide stripping filtration chambers. Gas stripping filters remove all dissolved gases in excess of the levels found in the air that flow through this filter. The primary purpose is to control carbon dioxide which will continue to accumulate in the culture system as the culture species respires and as wastes are broken down. Carbon dioxide is toxic in excess, but is a key input needed to produce phytoplankton. Therefore, air may flow through the gas stripping chamber of the main production sub-system (1) and potentially the zooplankton sub-system (3) and in both cases into the phytoplankton sub-system (2) as an input for photosynthesizing plankton.

#### 2. Phytoplankton sub-system (Figure C)

The phytoplankton sub-system (2) may be a similar tank and filter structure to the main production sub-system (1) with modifications that may maximize the following parameters. Ozonation or UV sterilization may not be necessary as the process will destroy algae cells. Research suggests ruptured algal cells may inhibit algal growth. Carbon dioxide removal is not necessary as the parameter is a limiting growth factor that should be maximized, not minimized. Depth, flow rate, and agitation may be minimized so that algal cells receive adequate light for photosynthesis but are not damaged. Gas infusion from production sub-system (1) gas strippers and water inflow from production sub-systems may provide

adequate carbon dioxide, nitrogenous fertilizers, and agitation. Carbon dioxide may be added to the phytoplankton sub-system by a method similar to that described for oxygen infusion in the production sub-system (1), and supplemental nitrogenous-based, phosphorous-based, and other fertilizers may be added to maximize algal growth. Algae from the micro-culture sub-system (11) may be used to seed the phytoplankton sub-system (2).

The information technology sub-system (8) services the phytoplankton sub-system (2) with similar methods and devices as those used in the production sub-system (1). However, the parameters may be monitored with different objectives because nitrogenous compounds and carbon dioxide are now inputs, not toxic by-products. Further, other parameters like pH may vary between sub-systems based on parameter levels that optimize growth as noted below. The key parameters that may need to be optimized in this example are levels of carbon dioxide, nitrate, and phosphorous, as well as light intensity throughout the water column. Too much or too little light may destroy or otherwise cause the death of algal cells which can contribute to the population crashing within the culture tank. A population crash is defined as a substantial amount of the culture species perishing, thereby inhibiting future growth under current conditions.

In this example, chlorella, and chlamydomonas algal strains are cultured, requiring the following conditions:

- 10,000 lux (full sunlight if dense)
- Temp: 10-20 good growth, 18-22 typically optimal, match other cultures
- pH8.2-8.7, 7-9
- CO2: .04% in air limiting, supplement in air injected at 0.5-2% filtered with air to 1μm before bubbled into cultures
- chlorella growth in 300L tank, >50million cells/ml after 130 hours
- 90g/m^3/day for continuous chemostat

One may increase growth in this system by supplements of carbon dioxide, nitrogen, and phosphorous. Transparent facility roofs may provide access to sunlight, but algal cultures may also be exposed to supplemental light sources. While this example may use natural sunlight, others should be tailored to the biological requirements of the algae, culture method, tank depth, and amount of circulation.

The phytoplankton subsystem (2) may consist of one culture tank or more than one, so long as adequate algal cells are available to support the zooplankton subsystem (3). If more than one tank is used, they may be maintained in various stages of algal bloom (population growth cycle) in order to fine-tune the production cycle. These tanks may be arranged vertically to allow water to flow through each tank into the next, with water originating from the main production sub-system (1). They may also be arranged horizontally in which water flow from the main production sub-system (1) may be diverted into any of the phytoplankton sub-system (2) culture tanks in any order, at any time, at either specified volumes over set intervals or as-needed. Water filtration may occur in this system through biological filter devices, though simple circulation by pump, water inflow, or gas inflow, may be sufficient. This system may be linked to the wastewater treatment sub-system (6) if substantial volumes of water need to be removed from the system. Such instances may occur if the algal population crashes, tanks need to be cleaned or reseeded from stored stocks (11), or for general maintenance.

Water and suspended algae may flow from the phytoplankton sub-system (2) into the zooplankton sub-system (3). This may occur when algae density in zooplankton culture tanks is low, or the algal population has reached a threshold density that if not reduced may lead to a population crash. The timing of water release from phytoplankton sub-systems (2) into zooplankton sub-systems (3), as well as inflows from production sub-systems (1) into phytoplankton sub-systems may be determined by the number of tanks used, the species cultured in

production, phytoplankton, and zooplankton subsystems, the growth rates of those species in that particular facility, and changes in the levels of water quality parameters. Hypothetical system proportions and parameters are given for the combinations of culture species following the system descriptions.

The flexibility of this system is a significant benefit of this design, and one that differs substantially from other intensive systems. This flexibility allows managers to modulate flows between systems so that water volumes, flow rates, and nutrient concentrations may be precisely controlled and stored for use in a different system or at a later point in the production time line.

Plant species such as duckweed may be cultured along with or in place of algae if managers wish to feed this directly to the production species, supplement feed pellets, stabilize water filtration, or employ its presence for other benefits.

Duckweed and other rapidly growing hardy plants commonly found in the ecosystems of final product species may benefit culture species nutritionally if fed, are easy to culture within intensive systems, and filter nitrogenous and other fertilizing system by-products including carbon dioxide from the water.

These production rates may require daily water flow from the phytoplankton subsystem (2) to the zooplankton sub-system (3). Water levels in phytoplankton (2) and zooplankton sub-systems (3) may be allowed to fluctuate to account for differences in flow rates between production (1) and phytoplankton (2), and zooplankton (3). This might occur if managers decide not to release water from the production sub-system (1) if sufficient levels of nitrate have not accumulated. These nitrate levels are a function of feed rates, amount of feed consumed, the levels of protein in the feed, rate of nitrification in the biological filter, exposure to air at the tank surface or in the gas stripping chamber, metabolic rates of production species, and other factors. A major benefit of this system is the increased flexibility and control over the complex interaction of these production

variables that attributes like sub-system separation, input regulation, and water volume modulation provide.

#### 3. Zooplankton sub-system (Figure D)

The zooplankton sub-system (3) resembles the production (1) and phytoplankton (2) sub-systems in design, devices, and methods used to culture the feed species. Like phytoplankton, zooplankton may grow better under reduced flow rates, so methods of water circulation, filtration, and water and gas inflow must be structured accordingly. For Daphnia in this example, water may be gently removed through a 100 µm screen in order to filter biologically, strip carbon dioxide, and oxygenate. Physical waste in the form of expired daphnia or ephippial egg cases may be removed at harvest, tank draining and cleaning, or other methods. One means of removing such waste through a bottom or top drain is to illuminate the tank away from the drain, which attracts the daphnia to the light source, and releasing water through the drain. Artificial illumination may be required for culture if sufficient light intensity does not exist through natural sources that may be accessed through a greenhouse roof on this portion of the physical plant.

In the zooplankton sub-system (2) of this example, the following parameters may be optimized around the following levels:

- 25C
- feed: 1 μm bacteria digested & assimilated w/ 50% efficiency, optimal nannoplanktonic algae esp. flagellates, not blue-greens, heterotrophic microflaggelates (up to paramecium size) excellent addition, detritus and benthic when food scarce, non-selective so high concentration suspended material detrimental to metabolism
- mass cultivation of daphnia magna on no/low-value agro-industrial residues, De Pauw et al., 1981
- 10<sup>5</sup> 10<sup>6</sup> algal cells/mL ideal, autotrophic increases system oxygen
- monoxenic or dixenic system of Chlorella, Chlamydomonas, Scenedesmus
- supplement w/ vitamin mix: in μg/L: Biotin 5 Thiamine 100,
   Pyridoxine 100, Pyridoxamine 3, Calcium Panthothenate 250, B12 (as

mannitol) 100, Nicotinic acid 50, Nicotinomide 50, Folic acid 20, Riboflavin 30, Inositol 90, Goulden et al., 1982→ add 1ml of this to each liter of culture water

- density: 20-100 animals/liter
- regular photoperiods
- temp: 15-25C
- maximize sodium and chloride, not toxic, control potassium and magnesium b/c toxic above 10mm and 30-240 µg/L respectively
- pH 7-8
- blooms: 100g/m<sup>3</sup> on cladocerans and manure, harvest 30% standing crop daily

The following or other methods may be used to transfer zooplankton sub-system (3) to the production sub-system (1):

Harvesting of the zooplankton species may occur by flowing water through a screen 600µm of sufficient size to only remove smaller members of the population. This may be the desired method if smaller organisms are used to feed live to fry or juvenile production cultures. In this case, the daphnia should not be removed from the water as exposure to gas may create gas pockets between the daphnid body and carapace, which will inhibit swimming and eventually result in death and may be harmful to production species if consumed. In some systems a more complex method of harvesting may not be necessary, but managers may want to ensure much of the algae suspended in the zooplankton culture tank has been consumed before flowing the zooplankton, water, and any remaining algae into a production culture tank. This method of harvesting may not be suitable for feeding to larger fish in production cultures.

Another method more appropriate for feeding fish that desire larger/adult daphnia is to submerge a 700  $\mu$ m screen or similar into the zooplankton culture tank, move the screen or allow the daphnia to migrate towards a light source, and drain water from the side of the partitioned culture tank that contains the larger daphnia. In this case, daphnia may be exposed to surface air or gas in the culture environment because adult finfish may be less sensitive to ingesting small

amounts of gas through their feed. Similar to the harvest method previously described, the zooplankton, water, and any remaining algae may flow into a production culture tank. Managers may want to limit the amount of algae suspended in the zooplankton sub-system (2) before flowing water into the production sub-system (3). If managers are concerned with minimizing the water exchange between sub-systems this method may not be appropriate.

A third method of harvesting which may be more useful when feeding larger production culture specimens or when nutritional supplements are added to the feed involves separating the zooplankton culture from the water in which it is cultured in, and potentially refined further. Daphnia may be skimmed directly from the zooplankton culture tank. Zooplankton may be flowed with water and remaining algae from the culture tank, collected with a screen of sufficiently small pore size to capture most or all daphnia. Daphnia may then be transferred from this screen by hand, water jet, squeegee, or other method and transported by conveyor belt or other method to the feed processing sub-system (4). The screen itself may be transferred to the feed processing subsystem (4) mechanically or manually. In this example, adult daphnia may provide a sufficiently large food particle to support goldfish growth, but this method may be employed if managers choose to increase food conversion ratios or growth rates by offering goldfish larger sized feed pellets or nutritionally enhanced pellets. However, since the nutritional attributes of daphnia correspond well to the dietary needs of goldfish, this method may not be necessary in this example. In this case, the option of separating daphnia from culture water previously described may not be necessary.

Other methods exist for segregating Daphnia by size within the zooplankton culture tanks. A screen, jointed in the middle, may be inserted in the center of the culture tank so that the two halves of the screen, each equal to the radius of the culture tank are folded and touching. Topically, this might resemble two hands of a clock at 12 and 1 o'clock. The "1 o'clock" hand may be moved

clockwise around the tank, allowing smaller daphnia to pass through, corralling larger daphnia between 11 o'clock and 12 o'clock, and might be removed from the system by a drain located between 11 and 12 o'clock. Light sources may be used to attract small and large daphnia at 5:30 and 11:30 respectively before removing larger daphnia from the culture tank.

A screen of sufficient-sized pores to trap adult daphnia and not juvenile daphnia may normally rest on the bottom of the culture environment, raised slowly to harvest adult daphnia, and returned to the bottom until the remaining daphnia mature. Managers may find maintaining daphnia of a variety of ages and sizes in a culture tank enhances growth rates, but because reproduction and growth are continuous and rapid, this may not be necessary for daphnia, but could be taken into consideration for other zooplankton species.

The previous harvesting methods may be employed within the system described in this example, and similar modifications may be made that do not differ substantially from the purpose and scope of the harvesting concept.

An additional benefit of extracting zooplankton species from their culture water before processing into feed is the ability to prevent water that contains parasites or is of sub-optimal water quality from entering the production culture environment.

#### 4. Feed processing sub-system

The feed processing sub-system (4) collects zooplankton and/or phytoplankton, non-planktonic feed sources such as filamentous algae, duckweed or other floating plants, zebra mussels, and any other live feed species capable of growth within the system through photosynthetic growth, chemosynthetic growth, or feeding on other system organisms. Different production species possess different nutritional requirements, and different species cultured as sources of

food are chosen based on their ability to meet these requirements and utilize nutrients within the system. Examples of species combinations, and reasons for selection are outlined after the description of this single-species freshwater system.

The method for refining feed species into feed for production species may involve forming a slurry from whole organisms, reducing water content, forming into pellets, drying through exposure to air and/or heat, and collecting for storage or transportation directly to the production culture sub-system (1). Managers may choose to include such nutritional supplements as amino acids, lipids, carbohydrates, vitamins, minerals, or other additives. These supplements may be added to the slurry.

A variety of other methods and devices may be employed to transform live feed into larger particles. Managers may decide upon these methods based on production requirements, size of facility, cost, desired level of control over food quality, storability of feed produced, and other factors. There should be no limitation on the actual selection of method and device that would depart from the claim that live feed is extracted from the culture tank and further processed into another form for feeding. Further, novel or otherwise undescribed methods of feed production should not be viewed as a departure from the protection of the claim (that live feed are removed from the system and processed to be used elsewhere in the system as a food input for production species or other use).

Finally, some feed species cultured may be of sufficient size for direct feeding into production systems. These might include larger copepods, other crustaceans resembling natural food size, or baitfish. These live feeds may still be removed from the system in which they are raised in order to be fed to production tanks in metered amounts and frequencies.

#### 5. Filtration sub-system

Four filtration methods may be used. Biological filters, as discussed earlier, provide a habitat for bacteria capable of reducing ammonia and nitrite to nitrate. Bacteria capable of decomposing organic matter may also reside in these filters. The purpose of such filtration is to reduce toxic nitrogenous wastes to non-toxic forms, so that they may be used in subsequent systems as inputs for photosynthetic organisms. A biological filter may resemble a cylinder in which water flows from the bottom through a fluidized bed of sand or other suspended media, to which ammonifying and nitrifying bacteria cling (see e.g., Goldman et al., Fluidized bed reactor and distribution system, patent # 5,330,652) Similarly, water may trickle down through a mosaic grid, to which these bacteria are attached. The first method uses space more efficiently, but may require supplemental oxygen, and may become dominated by decomposing bacteria. The latter method may require less maintenance and variable inputs, but requires significantly more space to convert the same amount of nitrogenous waste as the former system and may run the risk of difficult to remove physical matter if sufficient mechanical filtration is not present.

Ozone tubes may be used as a form of filtration. When ozone is injected into the water column at a specified atmospheric pressure and time the ozone will oxidize physical matter and chemicals in the water. The International Bottled Water Association exposes water to 1-2 mg/L ozone for 4-10 minutes. Production facilities will have to determine the exposure time and quantity based on their system that provides ozone, intended use, and production facility parameters. Ozone can be more effective than chlorine in destroying bacteria, fungi, and viruses, oxidizing organic and inorganic compounds as well as removal of iron and manganese. This may be useful for destroying parasites, and converting organic waste into carbon dioxide. The most common by-product of the ozone filter may be oxygen, as the ozone approaches a lower level of atmospheric

pressure. However, different by-products may be created as ozone interacts with a variety of chemicals dissolved in the water.

Mechanical filtration may occur by a variety of methods. Drum filters, screens, and any other method capable of separating solid waste, uneaten food, and any other physical particles from the culture media (water) may be used.

Gas-strippers, constructed through simple engineering or through the purchase of more costly devices may be used to separate dissolved gases from the water column. As mentioned earlier, as water trickles vertically down a porous mosaic, horizontal air flow may remove dissolved gas from the liquid, as the two states approach equilibrium concentrations of particular gases. For example, if there is more carbon dioxide in the culture water than there is in the air, the stripping process will increase the carbon dioxide concentration in the air and reduce it in the water. Similar changes will occur for each chemical in either the air that flows through the stripping chamber or those chemicals in the culture water. Higher concentrations will decrease, and lower concentrations will increase compared to the other state as gases approach equilibrium. This may be a non-selective process, so that managers may not easily control how much of a gas is transferred, unless the stripping device is precisely engineered and the air that is used to strip the gas changes based on the dissolved gas level in the culture tank and the desired concentration of gases for the phytoplankton sub-system (2).

#### 6. Solid waste removal and waste-water treatment sub-system

The purpose of the solid waste removal and water treatment sub-system may be threefold. First, solid waste is eliminated from culture tanks and stored. Once stored, managers, and plant designers may choose to either transport the waste offsite for agricultural fertilizer or other use, as either a cost or revenue. However, managers may also choose to recondition this solid waste to use internally as fertilizer. The degree of reconditioning depends on a variety of

factors. One might be to prevent the spread of harmful bacteria and parasites across systems or species. A marketing obstacle may be how managers market a final product that uses fish waste in an unaltered form as an input. However, given this waste is primarily used to grow plants for human consumption, this is less of an issue. The intensity of the system may dictate how finely waste should be processed. In more extensive systems, the added space required to use waste in its unaltered form may be useful, as it provides greater surface area for bacteria cultures that may serve as a food source for the subsequent zooplankton sub-system (3). Refining waste into chemical components and non-useable matter will allow managers to discard the less useful portion and have a source of nitrogenous and phosphorous-based waste that is easier to store and distribute and traditionally expensive to purchase.

#### 7. Final product processing sub-system

The final processing sub-system may be used to harvest fish or aquatic products from production tanks, pack with ice, clean and fillet or otherwise process for shipping and transform into a form consumers or wholesaler distributors desire. This system might connect to shipping bays and include refrigeration, freezing, employees or machines for cleaning, cutting, and packaging aquatic products.

#### 8. Information technology sub-system

The information technology sub-system may coordinate culture tanks, filters, and other systems by linking water probes and sensors with computer systems in order to monitor conditions, collect data, and/or notify controllers or automated input tanks to respond to changes in water quality and production. Probes in tanks may detect oxygen, carbon dioxide, nitrogen, temperature, pH, salinity, turbidity, and other system parameters. Information on system parameters may be stored in databases and analyzed, as well as instantly relayed to controllers or to such devices as oxygen or water storage tanks and release valves to optimize

culture environments. Maintaining dissolved oxygen levels to ensure the survival and optimal growth of culture species is a primary goal of this subsystem. Automating the regulation of other factors is a desirable option in the United States, where the cost of labor is high relative to other economies, and the best use of this technology may be to produce high-value niche products that compensate for the additional fixed costs associated with a capital-intensive system. These decisions are managerial, and plethora engineering modifications exist in order to implement this invention, but given the primary goal of the invention – to control and optimize system by-products for re-use as inputs in order to maximize the quality and volume of production while controlling costs – the practical application of this invention may benefit from increased investment in capital.

#### 9. Water storage sub-system

The Water storage sub-system may be used to store water collected from an exterior source such as a well, body of water, or river. The system may store water for treatment and/or release into culture tanks, food production, or wastewater or any other system. Treatment of water might include oxygenating, adjusting temperature, controlling salinity, as well as adjusting hardness. Different water systems may be used for different species based on the levels that maximize growth and/or health or other desired characteristics. Alternative storage tanks housing salts, heaters, or chillers may be attached to this system to adjust salinity or temperature.

#### 10. Ozone sub-system

The ozone sub-system may consist of pipes holding culture water at sufficient atmospheric pressure to inject ozone into the water. Water purification may be more effective under increased atmospheric pressure because the pressure may aid the dissolution of ozone gas into culture water. The system may link to the

information technology sub-system in order to regulate the flow of ozone entering the chamber in order to ensure adequate sterilization of water occurs but also that the level of ozone entering culture tanks does not reach a level toxic to fish. This system, along with other such sterilization methods as exposure to ultra-violet light or irradiation may be used to sterilize water in culture systems, waste-water treatment, food production or other systems.

#### 11. Micro-culture and live feed storage sub-system

In order to maintain a constantly available supply of live cultures, a sub-system may exist that stores these organisms. Other sub-systems that may require frequent seeding from sub-systems include the phytoplankton, zooplankton, and filter sub-systems. Algae, rotifers, and daphnia are examples of species maintained in this system. The sub-system may require precise monitoring of light, temperature, salinity, and other water quality parameters by the information technology sub-system or other method. As micro and larval cultures are frequently more sensitive to changes in water quality, system parameters may need to be held to small ranges. Further, feed inputs may need to be stored to supplement this system regularly. Additional husbandry techniques may need to be employed to ensure culture stock remain in good condition.

Other sub-systems may exist to grow larva to a size suitable for stocking in nurseries or production systems, or to breed and hatch broodstock in order to supply systems. The design of these systems may resemble production systems, though nutrition and water quality may need to be adjusted based on species requirements.

12. Broodstock sub-system. The design of the system may resemble production in form of tanks, filtration, feeding methods. However, broodstock are cultured for health, not growth, and for reproductive fecundity. Therefore, much benefit may be derived from the addition of live feed and optimal water conditions. This

system can also provide habitats for breeding, stripping and milt and eggs, or egg and fry rearing. The goal is to generate larva or juvenile production species for stocking in the production sub-system.

#### Multi-Species Embodiment

The single-species freshwater system described above separates and stores a variety of inputs at different stages of production. These inputs may be stored in excess of the needs for one system. Further, the process of separating these inputs from the culture environment requires a capital investment in plant, equipment, and the hiring of specialized labor. As output is expanded through larger plant designs, the cost per additional unit of output declines. Therefore, economies of scale and scope may be achieved by producing on a larger and broader scale. Additionally, because the system extracts specialized inputs, much benefit may be derived by expanding the production facility to produce a variety of species that may benefit from these inputs, and operate at a sufficiently high level of output to cover the fixed costs associated with extracting them, as well as the opportunity cost associated with purchasing these variable inputs from specialized distributors. The simple freshwater system previously described may be environmentally and energetically efficient, but may not produce fish at a profitable level on a small scale.

The costs associated with expanding the phytoplankton and zooplankton sub-systems decline on a per unit basis at higher levels of capacity. This is because the cost of physical and human capital needed to culture these organisms declines at a marginal rate as output expands. Additionally, finfish are tertiary feeders, requiring feed sources that are higher on the food chain than the food that filter feeders like mollusks require. Therefore, since the system already produces inputs such as planktonic algae, adding a mollusk culture system onto the phytoplankton sub-system may capitalize on the availability of inputs.

Fish raised in farms frequently die. Some farms save these fish in storage tanks and either sell, donate, or pay to have them removed, eventually being employed as fertilizer or destroyed. Since deceased fish are the natural food source of other commercially desirable final products, it may be logical to add production systems that rely on perished fish as a source of feed. For example, crab and crayfish cultures may be incorporated in this way. This combination of cultures reduces the need to remove dead fish from the facility and import additional food for scavengers.

Nori seaweed used for sushi wrappers, Ascophyllum nodosum and kelps for carrageenan and other emulsifiers, and diatoms used as polishers may be cultured in systems analogous to the phytoplankton sub-system. While this algae is benthic (attaches to a substrate) and the diatoms are planktonic, this system would fit into the plant in a similar position as phytoplankton cultured as a source of food elsewhere.

#### Combinations of Species Cultured

Different culture species have different environmental and nutritional requirements. Therefore, a farm rearing single or multiple species may maintain relatively consistent water conditions across production, phytoplankton, and zooplankton sub-systems. Consistent conditions may be a range of temperature, salinity, or water chemistry. While it may be possible to maintain significantly different environments among sub-systems within the same facility, it may be more cost effective to select species that require similar environments in order to reduce the costs associated with replicating those natural conditions. Therefore, species preferring similar temperature and salinity may be grouped together. Phytoplankton, zooplankton, and micro-cultures may be matched by environmental and nutritional requirements/characteristics, and taste of the final product species. For example, should a facility raise salmon as its final product, the farmer might cultivate Dunaliella and Rhodomonas algae, Tisbe japonicus copepods, and rotifers for the phytoplankton, zooplankton, and biofilters, respectively. Lobsters may be cultivated as a scavenger crustacean, and oysters as a mollusk. When selecting a scavenger

crustacean, the managers may want to weigh the costs of including that system compared with those for storing and disposing of dead salmon. Even if lobsters are slow to reach market size, the benefits of cultivating them may outweigh the costs mort storage and disposal, as well as foregone revenue.

The form of additional systems may resemble the final production sub-system (1), phytoplankton sub-system (2), or the zooplankton sub-system (3). Modifications may need to be made to fit the systems to different phyla. For example, mussels as a source of food or final product will need a substrate to attach themselves to. Therefore, it may be desirable to submerge a porous grid which maximizes the surface area of this substrate in the culture medium. Oysters and clams are frequently cultured in mesh bags to allow for water flow and provide ease of handling.

A variety of food species exist with which to feed final product species. Daphnia, moina, copepods, rotifers, baitfish, and zebra mussels are some examples based on growth rates, ease of cultivation, and nutritional value. The goal may be to shorten the food chain to concentrate nutrients per mass and reduce culture costs. Some of these inputs, like zebra mussels, may be useful to organisms that prefer feeding on mollusks, like striped bass, but may need to be refined for other final product species by pulverizing shells. Further, culturing mussels as a feed input may require calcium carbonate supplements to promote shell growth. The food and wastewater organisms that may best match combinations of product species and the required environmental conditions are listed below.

a) Haddock, flounder, halibut, salmon

lobster, dungeness crab

oyster

b) Striped bass, snapper, sea bass

blue crab, soft shell

oyster

c) ornamental tropicals

crustacean

sponges, urchin

a) tigriopus japonicus (calanoid), brachionus plicatilis (rotifer) chlorella, yeast

b) tisbe holothuriae

dunaliella, Rhodomonas

c) daphnia, moina

chlorella, chalmydomas

\* (percentages do not sum to 100 because U.S. capture and aquaculture figures include exports, whereas domestic demand does not)

- 1. Fish Stat Plus. FAO, Food and Agriculture Organization of the United Nations.
- 2. Source: BEA. NIPA accounts, year over year percent change, annual consumption of nondurable goods.
- 3. Economist. August.
- 4. Fish Stat Plus. FAO, Food and Agriculture Organization of the United Nations.
- 5. Fish Stat Plus. FAO, Food and Agriculture Organization of the United Nations.
- 6. Fish Stat Plus. FAO, Food and Agriculture Organization of the United Nations.
- 7. Fish Stat Plus. FAO, Food and Agriculture Organization of the United Nations.
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